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Review

Methylene blue: a controversial diagnostic acid and medication?

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A narrative review of the literature was conducted to determine if the administration of methylene blue (MB) in humans has potential risks. Studies were identified from MEDLINE, Web of Science, Scopus, and Cochrane. MB is a diagnostic substance used during some diagnostic procedures and also a part of the treatment of several diseases including methemoglobinemia, vasoplegic syndrome, fosfamide-induced encephalopathy, and cyanide intoxication, and the detection of leaks or position of parathyroid corpuscles during surgery. Although the use of MB is historically justified, and it ought to be safe, because it originated as a diagnostic material, the basic toxicological characteristics of this substance are unknown. Despite reports of severe adverse effects of MB, which could significantly exceed any possible benefits evaluated for the given indication. Therefore, the clinical use of MB currently represents a controversial problem given the heterogeneity of available data and the lack of preclinical data. This is in conflict with standards of safe use of such substances in human medicinal practice. The toxic effects of the application of MB are dose-dependent and include serious symptoms such as hemolysis, methemoglobinemia, nausea and vomitus, chest pain, dyspnoea, and hypertension. Some countries regard MB as harmful because of the resulting skin irritation and triggering of an adverse inflammatory response. MB induced serotoninergic toxicity clinically manifests as neuromuscular hyperactivity. This review aims to summarize the current understanding concerning the indications for MB administration and define the potential adverse effects of MB.

Key words: serotonin syndrome; neurotoxicity; methemoglobinemia; vasoplegic syndrome; methylene blue.

Introduction

Methylthioninium chloride, formally called methylene blue (MB), is an organic thiazine type compound with a dark blue-green color, crystalline structure and is markedly lipophilic. This substance was synthetically prepared for the textile manufacturer Heinrich Caro in 1876. Clinical practice and laboratory use of MB began only 14 years later.[1]

Molecular structure (Fig. 1) and physical and chemical properties define the pharmacokinetic profile of a substance. Within the physiological range of the gastric acidity (pH), MB is completely ionized.[2] Its bioavailability upon oral administration is 53–97%,[3] with plasma concentration peaking after 30–60 min[2–4] and a distribution volume value of 20 mL/kg.[2,5] This substance exhibits extracellular compartment kinetics with a terminal plasma half-life of 5–6 h.[4] Differences in organ distribution of MB are mainly responsible for the different pharmacokinetics after oral and intravenose (i.v.) administration. The principal sites of biotransformation are erythrocytes and peripheral tissues, where 65–85% of MB is metabolized to leucomethylthioninium chloride (leucomethylene blue), which is primarily eliminated by the kidneys and partly in the bile, along with the unmetabolized fraction, which gives urine and bile their characteristic colors.[6]

At the molecular level, MB has a broad range of mechanisms of action such as inhibition of the soluble guanyl cyclase, scavenging of nitric oxide (NO), and the modulation of the NO–cyclic guanosine monophosphate signaling pathway.[7] The biological effects are further enhanced by the synthesis of superoxide that interact with NO. This complex mode of action enables MB to dramatically reduce the concentration of a significant vasoactive intracellular factor. In addition to this principal biological target, it acts as a co-factor for (NADPH)-dependent methemoglobin reductase resulting in the formation of reduced methylthioninium chloride, which acts as an electron donor to reduce Fe^{3+} back to Fe^{2+}.[8] Deyes in general needs to be removed from nature due to their toxic effect.[9] Adsorptive removal of MB from synthetic water, using raw and acid-modified eucalyptus leaves.[10,11] MB removal through the process of adsorption has been a very popular study. Advantages and disadvantages of adsorbents, favorable conditions for particular adsorbate–adsorbent systems, and adsorption capacities of various low-cost adsorbents and commercial activated carbons as available in the literature are presented.[12–16]

We are here to present the usefulness of MB.

Pharmacological use of MB

MB is widely used in various fields, including biology, chemistry, and particularly medicine. Although many studies and case reports describe effective MB use, there are currently no precise guidelines concerning its safe use. One example is the use of MB in vasoplegic syndrome therapy, where this molecule has been applied for over 20 years without a clearly defined pharmacotoxicological frame of reference.[17]

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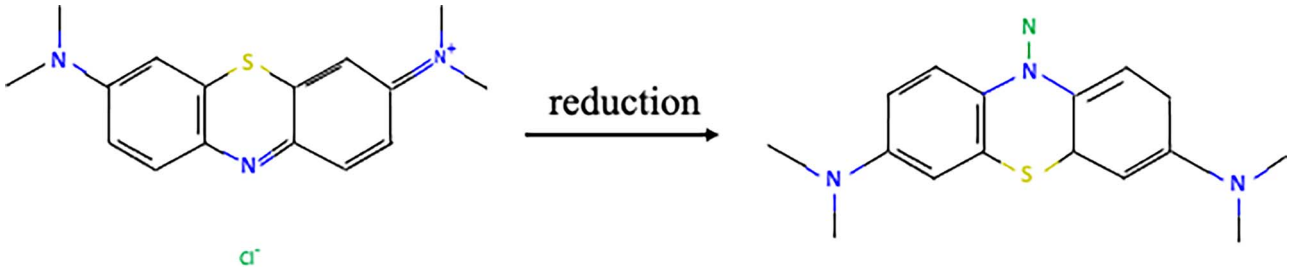

Fig. 1. MB molecular structure and its biotransformation to metabolite leucomethylene blue (MB is reduced to leucomethylene blue by methemoglobin reductases in erythrocytes) (pubchem.ncbi.nlm.nih.gov/compound/methylene_blue).

MB and vasoplegic syndrome

The vasodilatative shock secondary to cardiopulmonary bypass, characterized by low systemic vascular resistance with severe hypotension, tachycardia, normal or increased cardiac output and reduced pulmonary pressures is described as vasoplegic syndrome[18] or low systemic vascular resistance syndrome.[19] It is generally understood that this situation develops secondary to an inflammatory response initiated by extracorporeal blood circulation and is practically indistinguishable from septic shock,[20] where NO is produced and then released with a vasodilatative effect. This is a severe complication that would lead to the development of multiple organ failure unless adequate therapy is provided. In addition to the indicated inotropic medication, it is possible to administer so-called adjuvant medication, including vasopressin as well as MB,[21] which inhibits the effects of de novo synthesized NO. The required hemodynamic effect was reached after the infusion of 1% MB, 2 mg/kg, over 30 min and a second dose is given 22 h later.[22]

MB and methemoglobinemia

Methemoglobinemia induced by drug intoxication or other substances including cyanide, cocaine, carbon monoxide, leading to cyanosis, and/or unexplained low-oxygen saturation represents a significant patient risk of tissue hypoxia. Should the total methemoglobin concentration exceed 10%,[23] oxygen therapy application is deemed insufficient and aggressive management via MB medication commences.[8] The corresponding mechanism of action substantiates the use of MB, in this case leading to a rapid reduction of ferric state (Fe^{3+}) methemoglobin to ferrous state (Fe^{2+}) hemoglobin leading to improvement of binging oxygen followed by reducing tissues hypoxia. Therefore, MB represents a rapidly effective antidote of choice for symptomatic methemoglobinemia.[24]

MB and neuroprotection

MB has enabled of influence mitochondrial energetic metabolism in the absence of oxygen.[25] The mechanism potentiates sustained or increased adenosine triphosphate (ATP) production, thereby supporting cell survival.[26] Ongoing studies are trying to identify a molecule that would act neuroprotectively via an antioxidant mechanism for the treatment of various acute and chronic neurological disorders. Currently, there are no antioxidants that would radically slow down the progression of neurodegenerative diseases.[27–30] Preclinical tests have proven the protective effects of MB on cells in a pathophysiologic model of stroke, Parkinson's disease, and optic neuropathy.[31,32] Other studies present similar results from in vivo and in vitro studies.[33–36] MB also appears to be potentially useful for Alzheimer's disease therapy, where the benefit is provided from its antioxidative activity, facilitation of neurofibrillary tangles (tau proteins accumulations) degradation and clearance, promotion of autophagy, and reduction amylod plaques by positive influence on of β – amyloid protein levels.[37] The promising results from many studies support the hypothesis that MB and its derivatives could provide adequate efficacy in the therapy of diseases with etiopathogenesis dependent on caspase activation. MB has advanced to a phase 3 neuroprotection clinical study, concretely focused on Alzheimer's Disease.[38]

MB and cardiovascular conditions

MB reduces cyclic guanosine monophosphate (cGMP) concentration via guanylate cyclase inhibition (which causes relaxation of smooth muscle tissue of the vasculature) and thus has a vasoconstrictive effect.[39] Along with its ability to counteract the effect of NO, the complex image of MB action[22,40–42] is completed by mentioning its involvement in vasodilatation after an overdose of calcium channel blockers[43] or beta blockers.[44] Patients who do not respond sufficiently to standard treatment have the highest risks of developing this side effect owing to the chronic application of extremely high doses of calcium channel blockers or beta blockers.[42,45] MB-induced improvement of a hypotension state secondary to a vasodilatatory shock was described in cases of amlodipine[42,46] or quetiapine[44] intoxications and also as the result of an inappropriate combination of metformin and an angiotensin-converting-enzyme (ACE) inhibitor.[47]

MB and surgical intervention

MB is markedly lipophilic. This physico-chemical property allows its easy and rapid biodistribution across cell membranes and enables the temporary accumulation of MB inside cells. Direct application to mucosa for tissue visualization represents an everyday MB use in endoscopic diagnostics.[48–50] Intravenously administered MB will accumulate selectively in the parathyroid glands, which simplifies identification of these structures during surgical intervention.[51] This rather simple but exact method is also used to visualize other pathologically changed tissues by applying MB immediately before the given intervention. Additionally, MB is used to diagnose intragastric balloon rupture in patients undergoing endoscopic treatment of morbid obesity or for the detection of leaks following various surgical interventions.[52]

MB and septic shock

Critical care includes the management of potentially life-threatening disorders, mainly affecting vital organ functions. The most important syndrome treated in intensive care units is sepsis and septic shock. Sepsis is defined as a dysregulated systemic inflammatory response to infection. Septic shock is characterized by vasoplegia requiring the use of vasopressors (norepinephrine) to maintain adequate organ perfusion pressure.[53] The supposed

beneficial effect of MB is a decrease in smooth muscle relaxation by inhibition of NO production by inducible NO synthase (iNO). Moreover, MB directly reduces downstream activation of guanylate cyclase, an enzyme activated by NO.

To date, only 2 small and relatively old clinical randomized studies addressed the issue. Kirov et al.[54] included 20 patients with septic shock. Increased systemic vascular resistance measured as mean arterial pressure (MAP) in response to MB administration (i.v. bolus 2 mg/kg, followed by 0.25–2 mg/kg per hour for 24 h) compared to controls was observed. They also found a reduced dose of norepinephrine, epinephrine, and dobutamine, 87%, 81%, and 40%, respectively. Although statistically insignificant, the 28th-day survival in the MB group was 50% and 30% in the control group. No adverse effect was observed. Memis et al.[55] ($n = 30$) randomized into MB (0.5 mg/kg per hour) and control group (isotonic saline). The main aim was to determine plasma cytokine levels. However, they also observed significant increases in MAP in the study group without adverse effects.

The use of non-selective NO inhibition in the treatment of vasoplegia in critical care (i.e. L-arginine analogs) remains a matter of debate due to the possible adverse effects such as increased pulmonary vascular resistance and myocardial depression. A low-dose infusion rate might positively affect the risks.[52] MB as a low-cost selective iNOS inhibitor might seem to be a reasonable alternative to standard therapy of vasoplegia in both septic and non-septic vasoplegic distributive shock states. However, its definitive role is yet to be determined by further research.

MB an COVID-19

Coronavirus disease 2019 (COVID-19) is a severe worldwide pandemic increasing morbidity and mortality. Causal therapy is currently still lacking. The rationale for using MB in COVID-19 patients is based on antiviral activity inhibiting viral spike protein–ACE2 protein interaction,[56] direct inhibitory effects on iNO synthase (reducing the generation of reactive nitrogen species), and guanylate cyclase enzyme.[40] Moreover, MB acts as a potent oxygen superoxide scavenger,[57] prevents ROS production by inhibiting xanthine oxidase,[58] and decreases platelet activation, adhesion, and aggregation.[59]

In a recent randomized controlled trial, phase II, Hamidi-Alamdari et al.[60] randomly allocated 80 patients into 2 groups (Hammidi-Alamdari). MB was administered p.o. (1 mg/kg per 8 h for 2 days, then 1 mg/kg per 12 h for following 12 days) along with vitamin C, dextrose, and N-acetyl cysteine. The authors observed statistically significantly better oxygenation (pulse oximetry) in study group 3rd and 5th day of therapy ($P < 0.001$, and $P = 0.01$, respectively). Moreover, the MB group had a significantly lower respiratory rate, shortened hospital stay, and non-significantly improved 28th-day survival. The adverse effects of MB administration were rare, including vomiting ($n = 1$) and light headache ($n = 1$). No changes in consciousness, blood pressure, or dyspnea were observed. The authors proposed a reduced (colorless) form of MB as a promising, supplementary treatment of refractory hypoxemia in severe COVID-19 patients, which might also positively affect outcomes (Hammidi-Alamdari). A large multicenter randomized trial is, however still warranted.

MB adverse effects

The dose of MB varies significantly depending on the indication from 1 to 300 mg/kg daily dose. The toxic effects of the application of this substance are dose-dependent[61,62] and include serious symptoms such as hemolysis, paradoxic methemoglobinemia, nausea and vomitus, chest pain, dyspnoea, and hypertension. These adverse effects will manifest at doses exceeding 2–7 mg/kg.[63,64] Moreover, refractory hypotension and skin discoloration were documented upon administration of 20–80 mg/kg.[3]

Currently, there are only a few contraindication criteria.[65] One is glucose-6-phosphate dehydrogenase (G6PD) deficiency, where depletion of this enzyme in patients inhibits biotransformation of MB to leucomethylthioninium chloride, which the organism can eliminate. Thus, NADPH reductase deficiency concomitant with MB medication represents a significant risk of toxicity development.[66,67]

Preclinical pharmacokinetic studies on rodents have proven accumulation of MB in the central nervous system following intravenous administration.[4] In the last few years, clinical evidence has suggested that MB infusion during parathyroidectomy (5–10 mg/kg) causes protracted disorientation in the postoperative period.[68–75] These findings correlate with Nadler who already described data on vertigo, headache, tremor, and confusion accompanying the use of similar doses of MB.[76]

Serotonin syndrome as a consequence of MB use

MB has long been considered an inert dye, but its use in clinical practice has shown otherwise. MB is a reversible monoaminoxidase (MAO) inhibitor with a strong preference for the subtype A group.[77] Even a nanomolar quantity suffices to influence MAO-A function. The amount quoted in the literature as able to elicit a clinically relevant response is less than 1 mg/kg MB.[78] Recent data show that intravenous administration of 0.75 mg/kg MB leads to a plasmatic concentration of 500 ng/ml (1.6 μM), which, considering the lipophilic character of this substance, represents a sufficiently high concentration in the CNS leading to a substantial risk of MAO-A inhibition and a marked increase of intrasynaptic serotonin in the brain.[79] For this reason, MB may lead to severe side effects, particularly in the case of concomitant administration of serotoninergic substances, serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs).[80]

Methylthioninium chloride-induced serotoninergic toxicity clinically manifests as neuromuscular hyperactivity (tremor, myoclonus, and hyperreflection), and in an advanced stage as pyramidal rigidity and autonomous hyperactivity (diaphoresis, fever, tachycardia, tachypnea, mydriasis). Also, the mental state can be altered and the patient can feel agitated and excited. These symptoms are summarily described as the serotonin syndrome. Several deaths have been reported after a single concomitant administration of a MAO inhibitor and a SSRI category substance.[81–83] The number of deaths attributed to this concomitant administration is likely higher than reported because substances influencing neuronal serotonin level are widely used medications. Presently, MB is in use all over the world, even for indications other than visualization of the parathyroid corpuscles during surgical intervention and methemoglobinemia treatment (Table 1).

MB – induced cellular apoptosis and necrosis

MB increases inflammatory activity via the production of free oxygen radicals. This ability of inflammatory processes along with NO-induced vasodilatation, may result in a local toxic effect.[84,85] The cellular apoptosis rate in the compromised area depends on the exposition time and initiation dose.[86] Nevertheless, cases were reported of skin necrosis following an intravenous application of only 1% MB solution[87] or of submucosal ulceration and

Table 1. Clinical indications of MB and initiation of serotonin toxicity[a].

Clinical indication	Methylene blue dose	Serotonin toxicity
Methemoglobinemias	1–2 mg/kg i.v.[2]	No
Ifosfamide-induced encephalopathy	50 mg iv every 4 h until symptoms resolve	Yes
Treatment of vasoplegic syndrome	2 mg/ kg i.v.[2]	No
Parathyroid imaging	3–7.5 mg/kg i.v.[2]	Yes
Treatment of malaria	10 mg/kg twice a day orally for 3 days	No
Colonic diagnostic staining	200 mg single oral dose	No
Treatment of post-traumatic stress syndrome	260 mg orally for 6 days	No

[a]Adapted from Top, 2014[2] i.v.

necrosis of the colon secondary to MB use in laparoscopic colorectal surgery.[88] The development of cellular apoptosis falls within 2 h of MB administration, with a peak at 60 min.[89]

Local complications of MB contact are described as infection (5%), skin necrosis (1.25%), and skin hypersensitivity (.5%) based on the total number of monitored patients ($n = 398$).[90] Zakaria describes an incidence of local inflammatory infiltration after application of MB diluted to a 1:7 ratio.[91]

Hemodynamic effect of methylene blue

The correlation between MB application and its influence of coagulation factors[92] is based on its inhibitory activity of the NO/c-GMP signaling pathway, which mediates the activation[93] and aggregation[94] of thrombocytes and endothelial platelet adhesion. Thus, MB seems to have a pro-coagulation activity. Some literature sources explain these effects as result of the influencing of the tromboxan A2 production[95] and endothelial prostacycline I2 activity.[96] However, neither the relationship between inhibition of NO/cGMP signaling and inhibition of production of eicosanoids is currently clearly explained. Because tromboxan A2 (vasoconstriction and stimulation of platelet activation and aggregation) and prostacycline I2 (vasodilation and inhibition of platelet activation and aggregation) generally exert their action in an opposite manner, and the net effect of the inhibition of production of these eicosanoids is difficult to understand. For a patient within this paradigm, the risk consists namely in the possibility of the development of a thromboembolic complication, which is amplified by other factors such as an unsuitable combination of medications, obesity, insulin non-dependent diabetes mellitus, cancer, concurrent cardiovascular disease, or surgical procedure.

The controversy of methylene blue use in clinical practice

MB is the first phenothiazine type of chemical structure commonly used as a diagnostic and therapeutic substance for hereditary and toxonutritive methemoglobinemia. In these cases, MB is often the drug of choice.[24] However, there is a significant risk of the methemoglobinemia being induced by MB when it is administered at high doses or at standard doses to patients with renal insufficiency or G6PD deficit, or to pregnant females.[97] Genotypization of patients is typically not performed in clinical practice, although this marked risk may be associated with enzymatic depletion.

Pregnancy represents a relative contraindication of MB as it influences the production of NO in the placenta and fetus increasing risk of global or regional fetal hypoxia. On the other hand, MB administration is chosen for cases of neonatal refractory hypotension.[98] Even with this justified application, it is still necessary to consider the risk of a subsequent development of hemolytical anemia or hyperbilirubinemia in the newborn.[3]

The US Food and Drug Administration (FDA) also regulates the use of MB for other indications such as vasoplegic syndrome, ifosfamide-induced encephalopathy, and cyanide intoxication.[80] The use of MB for vasoplegic syndrome is still indicated despite the significant heterogeneity of clinical outcomes associated with the use of methylthioninium chloride for this purpose. Weiner et al.[99] reported that patients with vasoplegic syndrome treated with MB as a pharmacological rescue treatment exhibited higher postoperative morbidity and mortality. Despite this observation, the authors of this study point at the lack of toxicology data and recommend a strict review of the risk/benefit ratio prior to MB application, even in cases when it is not a first-choice medication.

This situation is further complicated by the fact that MB has a negative impact on arterial oxygenation.[17] The hypothesis regarding this phenomenon is based on the inhibition of NO-dependent vasodilatation in the systemic and pulmonary compartments and the potentiation of the vasoconstrictive effect of epinephrine.

Perioperative staining of the parathyroid glands by intravenously administered MB is presently supported by well-described methodology with documented efficacy, accuracy, simplicity, and safety of this application. Despite that, there is a rather large amount of data documenting postoperative neurological toxicity correlated with the administration of the described substance.[100]

In the past, the intrathecal application of MB used to be a common diagnostic procedure for cerebral ventricle visualization and the diagnosis of the cause of nosebleeds (rhinorrhea). This method has been abandoned because of an association between the administration of MB and the development of persistent cauda equina dysfunction or even a paraplegia lasting several hours.[101,102] Support for these observations was also provided by of Nadler in which healthy volunteers received 500 mg MB parenterally, followed by the appearance of prominent confusion for several hours.[76] The results of this study correspond with many others documenting MB-dependent encephalopathies associated with bolus MB doses or chronic SSRI medication.[68–75] However, these pathologies were not observed in patients treated with low MB doses.

A dependency between the dose and biological effect is discernible even regarding the induction of cellular death, which is very likely responsible for the neurological symptoms caused by MB.[89]

The risk of serotonin syndrome occurrence due to concomitant dosing of MB and SSRI, a representative group of common antidepressive medications, is probably the most important threat influencing the use of MB in clinical practice. Up to a several weeklong wash-out period is recommended in these patients to eliminate the risk of serotonin elevation in the CNS and allow the safe

use of MB. On the contrary, clinical data are available describing the antidepressive action of MB based on its ability to inhibit monoaminoxidase A, which plays a key role in the prevention of fosfamide encephalopathy.[77]

Conclusion

MB is a substance commonly used in diagnostic procedures and also for the treatment of several diseases such as methemoglobinemia, vasoplegic syndrome, fosfamide-induced encephalopathy, cyanide intoxication, and the detection of postsurgical leaks or position of parathyroid corpuscles during surgery. The maximum cumulative dose during a therapeutic cycle for methemoglobinemia is 7 mg/kg. However, applications of high doses or even recommended doses in certain patients are fraught with the risk of severe adverse events with undefined persistence and chronicity. The US FDA has issued a safety warning regarding the association of MB with the origin of severe neurological complications with not yet precisely known mechanisms of action. Based on the facts summarized herein, it is obvious that the clinical use of MB represents a rather controversial problem given the heterogeneity of available data and the lack of preclinical data, which is in conflict with standards of safe use of such substances in human medicinal practice.

Authors' contributions

Dr Bužga, Dr Machytka, Dr Dvořáčková, Dr Švagera, Dr Stejskal, Dr Máca researched and compiled available study data and assisted in the drafting of the manuscript. Concept and design: Dr Bužga, Drafting of the manuscript: Dr Bužga, Dr Machytka, Administrative, technical, or material support: Dr Král, Supervision: Dr Bužga.

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References

1. Wiklund L, Basu S, Miclescu A, Wiklund P, Ronquist G, Sharma HS. Neuro- and cardioprotective effects of blockade of nitric oxide action by administration of methylene blue. *Ann N Y Acad Sci*. 2007:**1122**(1):231–244.
2. DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs. I. Methylene blue–whole blood, urine, and tissue assays. *J Pharm Sci*. 1972:**61**(4):598–602.
3. Clifton J, Leikin JB. Methylene blue. *Am J Ther*. 2003:**10**(4):289–291.
4. Peter C, Hongwan D, Küpfer A, Lauterburg BH. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol*. 2000:**56**(3):247–250.
5. Srivastava A, Chaturvedi A, Gupta SK, Agarwal GR, Verma RK. Acute nitrobenzene poisoning: case fatality and importance of methylene blue. *Sri Lankan J Anaesthesiol*. 2010:**18**(2):91.
6. Rivero A, Muñoz R, Moreno M. What is your guess? *Clin Chem*. 2009:**55**(8):1600.
7. Graudins A, Lee HM, Druda D. Calcium channel antagonist and beta-blocker overdose: antidotes and adjunct therapies. *Br J Clin Pharmacol*. 2016:**81**(3):453–461.
8. Hunter L, Gordge L, Dargan PI, Wood DM. Methaemoglobinaemia associated with the use of cocaine and volatile nitrites as recreational drugs: a review. *Br J Clin Pharmacol*. 2011:**72**(1):18–26.
9. Sabnis RW. *Handbook of biological dyes and stains: synthesis and industrial applications*. 1st ed. John Wiley & Sons, Inc.; 2010
10. Ghosh K, Bar N, Biswas AB, Das SK. Elimination of crystal violet from synthetic medium by adsorption using unmodified and acid-modified eucalyptus leaves with MPR and GA application. *Sustain Chem Pharm*. 2021:**19**:100370.
11. Vermeulen T. Theory for irreversible and constant-pattern solid diffusion. *Ind Eng Chem*. 1953:**45**(8):1664–1670.
12. Gupta VK, Suhas null. Application of low-cost adsorbents for dye removal–a review. *J Environ Manag*. 2009:**90**(8):2313–2342.
13. Khodaie M, Ghasemi N, Moradi B, Rahimi M. Removal of methylene blue from wastewater by adsorption onto $ZnCl_2$ activated corn husk carbon equilibrium studies. *J Chem*. 2013:**2013**:e383985.
14. Ghosh I, Kar S, Chatterjee T, Bar N, Das SK. Removal of methylene blue from aqueous solution using *Lathyrus sativus* husk: adsorption study, MPR and ANN modelling. *Process Saf Environ Prot*. 2021:**149**:345–361.
15. Bae HJ, Encinar MP, Lozano-Durán A. Causal analysis of self-sustaining processes in the logarithmic layer of wall-bounded turbulence. *J Phys Conf Ser*. 2018:**1001**:012013.
16. Ghosh K, Bar N, Biswas AB, Das SK. Removal of methylene blue (aq) using untreated and acid-treated eucalyptus leaves and GA-ANN modelling. *Can J Chem Eng*. 2019:**97**(11):2883–2898.
17. Evora PRB, Alves Junior L, Ferreira CA, et al. Twenty years of vasoplegic syndrome treatment in heart surgery. Methylene blue revised. *Rev Bras Cir Cardiovasc Orgao Of Soc Bras Cir Cardiovasc*. 2015:**30**(1):84–92.
18. Gomes WJ, Carvalho AC, Palma JH, Gonçalves I, Buffolo E. Vasoplegic syndrome: a new dilemma. *J Thorac Cardiovasc Surg*. 1994:**107**(3):942–943.
19. Myles PS, Leong CK, Currey J. Endogenous nitric oxide and low systemic vascular resistance after cardiopulmonary bypass. *J Cardiothorac Vasc Anesth*. 1997:**11**(5):571–574.
20. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. *J Cardiothorac Vasc Anesth*. 1997:**11**(3):355–366.
21. Shanmugam G. Vasoplegic syndrome–the role of methylene blue. *Eur J Cardio-Thorac Surg Off J Eur Assoc Cardio-Thorac Surg*. 2005:**28**(5):705–710.
22. Pagni S, Austin EH. Use of intravenous methylene blue for the treatment of refractory hypotension after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. 2000:**119**(6):1297–1298.
23. D'sa SR, Victor P, Jagannati M, Sudarsan TI, Carey R, a. B, Peter JV. Severe methemoglobinemia due to ingestion of toxicants. *Clin Toxicol Phila Pa*. 2014:**52**(8):897–900.
24. Kakhandki S, Yahya M, Praveen M. Acute methaemoglobinaemia initially treated as organophosphate poisoning leading to atropine toxicity. *Indian J Anaesth*. 2012:**56**(4):397–400.
25. Rojas JC, Bruchey AK, Gonzalez-Lima F. Neurometabolic mechanisms for memory enhancement and neuroprotection of methylene blue. *Prog Neurobiol*. 2012:**96**(1):32–45.
26. Shen Q, Du F, Huang S, Rodriguez P, Watts LT, Duong TQ. Neuroprotective efficacy of methylene blue in ischemic stroke: an MRI study. *PLoS One*. 2013:**8**(11):e79833.
27. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev*. 2008:(2):CD007176.
28. Laurin D, Foley DJ, Masaki KH, White LR, Launer LJ. Vitamin E and C supplements and risk of dementia. *JAMA*. 2002:**288**(18):2266–2268.
29. Kamat CD, Gadal S, Mhatre M, Williamson KS, Pye QN, Hensley K. Antioxidants in central nervous system diseases: preclinical

promise and translational challenges. *J Alzheimers Dis JAD*. 2008:**15**(3):473–493.
30. Delport A, Harvey BH, Petzer A, Petzer JP. Methylene blue and its analogues as antidepressant compounds. *Metab Brain Dis*. 2017:**32**(5):1357–1382.
31. Wen Y, Li W, Poteet EC, et al. Alternative mitochondrial electron transfer as a novel strategy for neuroprotection. *J Biol Chem*. 2011:**286**(18):16504–16515.
32. Rojas JC, John JM, Lee J, Gonzalez-Lima F. Methylene blue provides behavioral and metabolic neuroprotection against optic neuropathy. *Neurotox Res*. 2009:**15**(3):260–273.
33. Poteet E, Winters A, Yan LJ, et al. Neuroprotective actions of methylene blue and its derivatives. *PLoS One*. 2012:**7**(10): e48279.
34. van der Plaat LW, Bulstra GH, Albers GHR, Eerenberg JP, van der Vis HM. Treatment of an osteoporotic vertebral compression fracture with the StaXx FX system resulting in intrathoracic wafers: a serious complication. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc*. 2012:**21**(Suppl 4):S445–S449.
35. Yoo AJ, Sheth KN, Kimberly WT, et al. Validating imaging biomarkers of cerebral edema in patients with severe ischemic stroke. *J Stroke Cerebrovasc Dis Off J Natl Stroke Assoc*. 2013:**22**(6):742–749.
36. Gomes B, Higginson IJ, Calanzani N, et al. Preferences for place of death if faced with advanced cancer: a population survey in England, Flanders, Germany, Italy, the Netherlands, Portugal and Spain. *Ann Oncol Off J Eur Soc Med Oncol*. 2012:**23**(8):2006–2015.
37. Appleby BS, Nacopoulos D, Milano N, Zhong K, Cummings JL. A review: treatment of Alzheimer's disease discovered in repurposed agents. *Dement Geriatr Cogn Disord*. 2013:**35**(1–2): 1–22.
38. Pakavathkumar P, Sharma G, Kaushal V, Foveau B, LeBlanc AC. Methylene blue inhibits caspases by oxidation of the catalytic cysteine. *Sci Rep*. 2015:**5**:13730.
39. Saha BK, Burns SL. The story of nitric oxide, sepsis and methylene blue: a comprehensive pathophysiologic review. *Am J Med Sci*. 2020:**360**(4):329–337.
40. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. *Biochem Pharmacol*. 1993:**45**(2): 367–374.
41. Marczin N, Ryan US, Catravas JD. Methylene blue inhibits nitrovasodilator- and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells via generation of superoxide anion. *J Pharmacol Exp Ther*. 1992:**263**(1):170–179.
42. Aggarwal N, Kupfer Y, Seneviratne C, Tessler S. Methylene blue reverses recalcitrant shock in β-blocker and calcium channel blocker overdose. *BMJ Case Rep*. 2013:**2013**:bcr2012007402.
43. St-Onge M, Dubé PA, Gosselin S, et al. Treatment for calcium channel blocker poisoning: a systematic review. *Clin Toxicol Phila Pa*. 2014:**52**(9):926–944.
44. Fisher J, Taori G, Braitberg G, Graudins A. Methylene blue used in the treatment of refractory shock resulting from drug poisoning. *Clin Toxicol Phila Pa*. 2014:**52**(1):63–65.
45. Burkes R, Wendorf G. A multifaceted approach to calcium channel blocker overdose: a case report and literature review. *Clin Case Rep*. 2015:**3**(7):566–569.
46. Jang DH, Nelson LS, Hoffman RS. Methylene blue in the treatment of refractory shock from an amlodipine overdose. *Ann Emerg Med*. 2011:**58**(6):565–567.
47. Plumb B, Parker A, Wong P. Feeling blue with metformin-associated lactic acidosis. *BMJ Case Rep*. 2013:**2013**:bcr2013008855.
48. Bruno MJ. Magnification endoscopy, high resolution endoscopy, and chromoscopy; towards a better optical diagnosis. *Gut*. 2003:**52**(Suppl 4):iv7–iv11.
49. Kiesslich R, Neurath MF, Galle PR. Chromoendoscopy and magnifying endoscopy in patients with gastroesophageal reflux disease. Useful or negligible? *Dig Dis Basel Switz*. 2004:**22**(2):142–147.
50. Marana R, Catalano GF, Muzii L. Salpingoscopy. *Curr Opin Obstet Gynecol*. 2003:**15**(4):333–336.
51. Dudley NE. Methylene blue for rapid identification of the parathyroids. *Br Med J*. 1971:**3**(5776):680–681.
52. Kwok ESH, Howes D. Use of methylene blue in sepsis: a systematic review. *J Intensive Care Med*. 2006:**21**(6):359–363.
53. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016:**315**(8):801–810.
54. Kirov MY, Evgenov OV, Evgenov NV, et al. Infusion of methylene blue in human septic shock: a pilot, randomized, controlled study. *Crit Care Med*. 2001:**29**(10):1860–1867.
55. Memis D, Karamanlioglu B, Yuksel M, Gemlik I, Pamukcu Z. The influence of methylene blue infusion on cytokine levels during severe sepsis. *Anaesth Intensive Care*. 2002:**30**(6):755–762.
56. Bojadzic D, Alcazar O, Buchwald P. Methylene blue inhibits the SARS-CoV-2 spike–ACE2 protein-protein interaction–a mechanism that can contribute to its antiviral activity against COVID-19. *Front Pharmacol*. 2021:**11**. [accessed 2022 February 10]. https://www.frontiersin.org/article/10.3389/fphar.2020.600372.
57. Riedel W, Lang U, Oetjen U, Schlapp U, Shibata M. Inhibition of oxygen radical formation by methylene blue, aspirin, or alpha-lipoic acid, prevents bacterial-lipopolysaccharide-induced fever. *Mol Cell Biochem*. 2003:**247**(1–2):83–94.
58. Salaris SC, Babbs CF, Voorhees WD. Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. *Biochem Pharmacol*. 1991:**42**(3):499–506.
59. Miclescu A, Wiklund L. Methylene blue, an old drug with new indications? *Jurnalul Roman Anestezie Ter Intensiv J Anaesth Intensive Care*. 2010:**17**(1):35–41.
60. Hamidi-Alamdari D, Hafizi-Lotfabadi S, Bagheri-Moghaddam A, et al. Methylene blue for treatment of hospitalized COVID-19 patients: a randomized, controlled, open-label clinical trial, phase 2. *Rev Investig Clin Organo Hosp Enfermedades Nutr*. 2021:**73**(3):190–198.
61. Ozal E, Kuralay E, Yildirim V, et al. Preoperative methylene blue administration in patients at high risk for vasoplegic syndrome during cardiac surgery. *Ann Thorac Surg*. 2005:**79**(5):1615–1619.
62. Evora PR. Should methylene blue be the drug of choice to treat vasoplegias caused by cardiopulmonary bypass and anaphylactic shock? *J Thorac Cardiovasc Surg*. 2000:**119**(3):632–634.
63. Liao YP, Hung DZ, Yang DY. Hemolytic anemia after methylene blue therapy for aniline-induced methemoglobinemia. *Vet Hum Toxicol*. 2002:**44**(1):19–21.
64. Harvey JW, Keitt AS. Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinaemia. *Br J Haematol*. 1983:**54**(1):29–41.
65. McDonagh EM, Bautista JM, Youngster I, Altman RB, Klein TE. PharmGKB summary: methylene blue pathway. *Pharmacogenet Genomics*. 2013:**23**(9):498–508.

66. Müller O, Meissner P, Mansmann U. Glucose-6-phosphate dehydrogenase deficiency and safety of methylene blue. *Drug Saf*. 2012:**35**(1):85 author reply 85-86.
67. Youngster I, Arcavi L, Schechmaster R, et al. Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence-based review. *Drug Saf*. 2010:**33**(9):713–726.
68. Bach KK, Lindsay FW, Berg LS, Howard RS. Prolonged post-operative disorientation after methylene blue infusion during parathyroidectomy. *Anesth Analg*. 2004:**99**(5):1573–1574.
69. Kartha SS, Chacko CE, Bumpous JM, Fleming M, Lentsch EJ, Flynn MB. Toxic metabolic encephalopathy after parathyroidectomy with methylene blue localization. *Otolaryngol–Head Neck Surg Off J Am Acad Otolaryngol-Head Neck Surg*. 2006:**135**(5):765–768.
70. MAS K, North AP, Chadwick DR. Prolonged postoperative altered mental status after methylene blue infusion during parathyroidectomy: a case report and review of the literature. *Ann R Coll Surg Engl*. 2007:**89**(2):W9–W11.
71. Majithia A, Stearns MP. Methylene blue toxicity following infusion to localize parathyroid adenoma. *J Laryngol Otol*. 2006:**120**(2):138–140.
72. Martindale SJ, Stedeford JC. Neurological sequelae following methylene blue injection for parathyroidectomy. *Anaesthesia*. 2003:**58**(10):1041–1042.
73. Mathew S, Linhartova L, Raghuraman G. Hyperpyrexia and prolonged postoperative disorientation following methylene blue infusion during parathyroidectomy. *Anaesthesia*. 2006:**61**(6):580–583.
74. Mihai R, Mitchell EW, Warwick J. Dose-response and postoperative confusion following methylene blue infusion during parathyroidectomy. *Can J Anaesth J Can Anesth*. 2007:**54**(1):79–81.
75. Sweet G, Standiford SB. Methylene-blue-associated encephalopathy. *J Am Coll Surg*. 2007:**204**(3):454–458.
76. Nadler JE, Green H, Rosenbaum A. Intravenous injection of méthylène blue in man with reference to its toxic symptoms and effect on the electrocardiogram. *Am J Med Sci*. 1934:**188**(1):15–21. accessed 2022 January 24. https://www.cabdirect.org/cabdirect/abstract/19352900449.
77. Ramsay RR, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol*. 2007:**152**(6):946–951.
78. Schwiebert C, Irving C, Gillman PK. Small doses of methylene blue, previously considered safe, can precipitate serotonin toxicity. *Anaesthesia*. 2009:**64**(8):924.
79. Gillman PK. CNS toxicity involving methylene blue: the exemplar for understanding and predicting drug interactions that precipitate serotonin toxicity. *J Psychopharmacol Oxf Engl*. 2011:**25**(3):429–436.
80. Research C for DE and. FDA drug safety communication: serious CNS reactions possible when methylene blue is given to patients taking certain psychiatric medications. *FDA*. Published online 2019 June 28 [accessed 2022 January 24]. https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-serious-cns-reactions-possible-when-methylene-blue-given-patients.
81. Héritier Barras AC, Walder B, Seeck M. Serotonin syndrome following methylene blue infusion: a rare complication of antidepressant therapy. *J Neurol Neurosurg Psychiatry*. 2010:**81**(12):1412–1413.
82. Cassens S, Nickel EA, Quintel M, Neumann P. The serotonin syndrome. Fatal course of intoxication with citalopram and moclobemide. *Anaesthesist*. 2006:**55**(11):1189–1196.
83. Zonneveld AM, Hagenaars M, Voermans NC, Gelissen HPMM, Claassen J, a. HR. Life-threatening serotonin syndrome following a single dose of a serotonin reuptake inhibitor during maintenance therapy with a monoamine oxidase inhibitor. *Ned Tijdschr Geneeskd*. 2006:**150**(19):1081–1084.
84. Garthwaite G, Garthwaite J. Cyclic GMP and cell death in rat cerebellar slices. *Neuroscience*. 1988:**26**(1):321–326.
85. Lee YS, Wurster RD. Methylene blue induces cytotoxicity in human brain tumor cells. *Cancer Lett*. 1995:**88**(2):141–145.
86. Werner I, Guo F, Bogert NV, et al. Methylene blue modulates transendothelial migration of peripheral blood cells. *PLoS One*. 2013:**8**(12):e82214.
87. Polito A, Parisini E, Ricci Z, Picardo S, Annane D. Vasopressin for treatment of vasodilatory shock: an ESICM systematic review and meta-analysis. *Intensive Care Med*. 2012:**38**(1):9–19.
88. Mebazaa A, Pitsis AA, Rudiger A, et al. Clinical review: practical recommendations on the management of perioperative heart failure in cardiac surgery. *Crit Care Lond Engl*. 2010:**14**(2):201.
89. Vutskits L, Briner A, Klauser P, et al. Adverse effects of methylene blue on the central nervous system. *Anesthesiology*. 2008:**108**(4):684–692.
90. Lee JH, Chang CH, Park CH, Kim JK. Methylene blue dye-induced skin necrosis in immediate breast reconstruction: evaluation and management. *Arch Plast Surg*. 2014:**41**(3):258–263.
91. Zakaria S, Hoskin TL, Degnim AC. Safety and technical success of methylene blue dye for lymphatic mapping in breast cancer. *Am J Surg*. 2008:**196**(2):228–233.
92. Johnstone MT, Lam JY, Lacoste L, Baribeau J, Théroux P, Waters D. Methylene blue inhibits the antithrombotic effect of nitroglycerin. *J Am Coll Cardiol*. 1993:**21**(1):255–259.
93. Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta*. 1993:**1178**(2):153–175.
94. Schafer AI, Alexander RW, Handin RI. Inhibition of platelet function by organic nitrate vasodilators. *Blood*. 1980:**55**(4):649–654.
95. Schrör K, Grodzinska L, Darius H. Stimulation of coronary vascular prostacyclin and inhibition of human platelet thromboxane A2 after low-dose nitroglycerin. *Thromb Res*. 1981:**23**(1–2):59–67.
96. Salvemini D, Currie MG, Mollace V. Nitric oxide-mediated cyclooxygenase activation. A key event in the antiplatelet effects of nitrovasodilators. *J Clin Invest*. 1996:**97**(11):2562–2568.
97. Walter-Sack I, Rengelshausen J, Oberwittler H, et al. High absolute bioavailability of methylene blue given as an aqueous oral formulation. *Eur J Clin Pharmacol*. 2009:**65**(2):179–189.
98. Driscoll W, Thurin S, Carrion V, Steinhorn RH, Morin FC. Effect of methylene blue on refractory neonatal hypotension. *J Pediatr*. 1996:**129**(6):904–908.
99. Weiner MM, Lin HM, Danforth D, Rao S, Hosseinian L, Fischer GW. Methylene blue is associated with poor outcomes in vasoplegic shock. *J Cardiothorac Vasc Anesth*. 2013:**27**(6):1233–1238.
100. Kadoya T, Kinoshita Y, Shiraishi M, Uehara H, Yamamoto T, Suetsugu K. Seven cases of parathyroidectomy for secondary hyperparathyroidism using methylene blue: suggestion for the method of methylene blue infusion. *Masui*. 2014:**63**(8):862–865.
101. Evans JP, Keegan HR. Danger in the use of intrathecal methylene blue. *JAMA*. 1960:**174**:856–859.
102. Arieff AJ, Pyzik SW. Quadriplegia after intrathecal injection of methylene blue. *J Am Med Assoc*. 1960:**173**:794–796.